Impact of a Chytrid-related mortality event on a population of the Green and Golden Bell Frog *Litoria aurea*

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ABSTRACT

Chytridiomycosis is considered a key threatening process under Commonwealth legislation. Little is known of the impact of this pathogen within wild populations. The Green and Golden Bell Frog *Litoria aurea* is a threatened species that is thought to be affected by this disease. Here we present an example of a chytrid related mortality event within an apparently healthy population of this species. The individuals were found to be carrying chytrid and we suggest that this was the proximal cause of mortality. Monitoring was conducted across Sydney Olympic Park between 1998 and 2005. In a small group of ponds in 1999, 23 individuals were found dead with 17 of these confirmed to have died as a result of chytrid infection within a short period of time. However, the number of frogs located within the complex remained at similar levels for the duration of the monitoring program. Management of wild populations of the Green and Golden Bell Frog should maximise the area of available habitat for this species and the connectivity between habitats in an attempt to minimise the impact of disease outbreaks.

Key words: *Litoria aurea*, green and golden bell frog, Chytridiomycosis, chytrid fungus, Sydney Olympic Park

Introduction

Chytridiomycosis has been considered to be the proximal cause of population declines in a large number of amphibian species worldwide (e.g. Lips *et al.* 2004; Weldon *et al.* 2004; Hero and Morrison 2005). As a result *Infection of frogs by amphibian chytrid causing the disease chytridiomycosis* has been listed as a key threatening process under the Australian Commonwealth *Environmental Protection and Biodiversity Conservation Act* 1999, as well as under the NSW *Threatened Species Conservation Act* 1995. However, little is known about the impact this disease has on wild populations that haven't become locally extinct (although see Burgin *et al.* 2005; Kriger and Hero 2006).

The Green and Golden Bell Frog *Litoria aurea* is a threatened frog species in south-eastern Australia. Historically, the species was found from East Gippsland in Victoria to Byron Bay in New South Wales (Goldingay 1996) and inland as far as Bathurst, Armidale (White and Pyke 1996) and the southern Tablelands (Osborne *et al.* 1996). Since the late 1960's, the species has undergone declines throughout its range, resulting in the species becoming largely coastal in its distribution (White and Pyke 1996).

No single factor has been demonstrated conclusively as being responsible for the observed decline of the Green and Golden Bell Frog. Several factors have been proposed, including predation by the introduced plague minnow *Gambusia holbrooki* (White and Pyke 1996; Morgan and Buttemer 1996; Hamer *et al.* 2002), habitat alteration (Goldingay 1996; Osborne *et al.*1996; White and Pyke 1996) and increasing UV-B radiation (Osborne *et al.* 1996; van de Mortal and Buttemer 1996). Chytrid fungus has also been found in a number of populations of this species and some have argued it may be the main cause of the observed declines of the Green and Golden Bell Frog (DEC 2005).

Little is known about the extent to which chytrid fungus impacts upon populations of the Green and Golden Bell Frog. Given the catastrophic impact the disease is thought to have had on other anurans (Berger *et al.* 1998; Berger *et al.* 1999), comparative information would be valuable for the conservation and management of the Green and Golden Bell Frog. Here we present an example of how a component of the Sydney Olympic Park population of the Green and Golden Bell Frog responded to a mortality event attributed to the chytrid fungus. From this information we consider factors that may have enabled the population to survive the event and identify areas for future research and management.

Methods

The study was conducted at Sydney Olympic Park (the site), situated 10 km west of the Sydney CBD. Data presented in this paper are part of the Green and Golden Bell Frog monitoring program at this site during the period from 1998 through to 2005. The site contains 425 ha of parkland of which approximately 200 ha are

classified as habitat for the Green and Golden Bell Frog. The mortality event was recorded in a small group of constructed ponds in the Kronos Hill precinct covering an area of 4 ha, known as the GE (Gabion East) complex to park managers. Within the GE complex there is one semi-permanent pond and seven ephemeral ponds (Figure 1). The semi-permanent pond is approximately 20 m long and 5 m wide, with 50% cover of macrophytes and 50% open water. Ephemeral ponds vary in size from approximately 2 m by 2 m up to approximately 10 m by 10 m. These ponds vary in the cover of macrophytes from 0 to 90%.

Frogs were located during nocturnal spotlight transects in and around habitat ponds at approximately six weekly intervals between the months of November and May. During these surveys, counts were made of the number of visible and calling adult and juvenile Green and Golden Bell Frogs. Habitat areas that were searched included all aquatic vegetation (primarily *Typha orientalis* and *Juncus* spp.), open water sections, vegetated and bare earth sections of the pond edge and the terrestrial grass areas within 5 m of the pond edge and those between individual ponds. All surveys

Figure 1. Sydney Olympic Park, with an insert of the GE complex where the mortality event occurred.

were conducted in accordance with the New South Wales National Parks & Wildlife Service frog hygiene protocol (NPWS 2000).

The ponds within the GE complex and the surrounding areas were surveyed by two people for a total of 90 minutes. Surveys were conducted in the time between 1 hour after sunset and 0400 hours. Similar survey effort was applied to all habitat ponds across Sydney Olympic Park. Any dead or dying frogs observed during the nocturnal spotlight transects were collected in sterile plastic bags and taken to the Veterinary Quarantine Centre, Taronga Zoo, for a post mortem. Histopathology was used to determine the presence of the chytrid fungus and an assessment was made as to whether it was the likely cause of death. Methods for testing chytrid fungus now include a higher degree of certainty and the ability to test live animals. These techniques were not available to us at the time of the study and therefore we are cautious in our treatment of the results.

Results

Throughout the monitoring program, generally less than 10 animals were observed in the GE complex (Figure 2). During only eight of the 55 surveys conducted at these sites were more than 10 animals observed. The highest number of frogs encountered occurred at the same time as the mortality event (April 1999). It is thought that the low numbers recorded in the 2003 and 2004 surveys were related to the drought occurring at that time during which there was little or no water in all of these ponds. There was considerable variation in the numbers of frogs encountered in the GE complex over time, however, the range of this variation does not appear to have reduced since the mortality event (Figure 2). It is possible that these fluctuations are due to meteorological changes, but these data are not readily available and are beyond the scope of the paper.

In the autumn surveys of 1999, a total of 23 Green and Golden Bell Frogs were found dead or dying, in and around the GE complex. No dead or dying frogs were found in other areas of the site at this time despite a consistent survey effort across the site. Red leg was considered the cause of death in two cases (K. Rose, Taronga Zoo pathologist, *pers. comm.* 1999), however, the cause of death for the remaining 15 could not be determined. Interestingly, no dead individuals of the five other frog species found at the site were located. Although this may be a function of the surveys being designed to target the Green and Golden Bell Frog.

Chytridiomycosis was given as the cause of death in 17 of the autumn 1999 cases (4 male; 6 female; 7 sex not recorded). Of the remaining cases, two other deaths were attributed to red leg (1 male; 1 female), one to epidermitis (1 female) and the cause of death could not be determined for three cases (1 male; 2 sex not recorded). Throughout the seven years of the monitoring program, 17 other dead Green and Golden Bell Frogs were located with no more than five deaths recorded in any one survey period

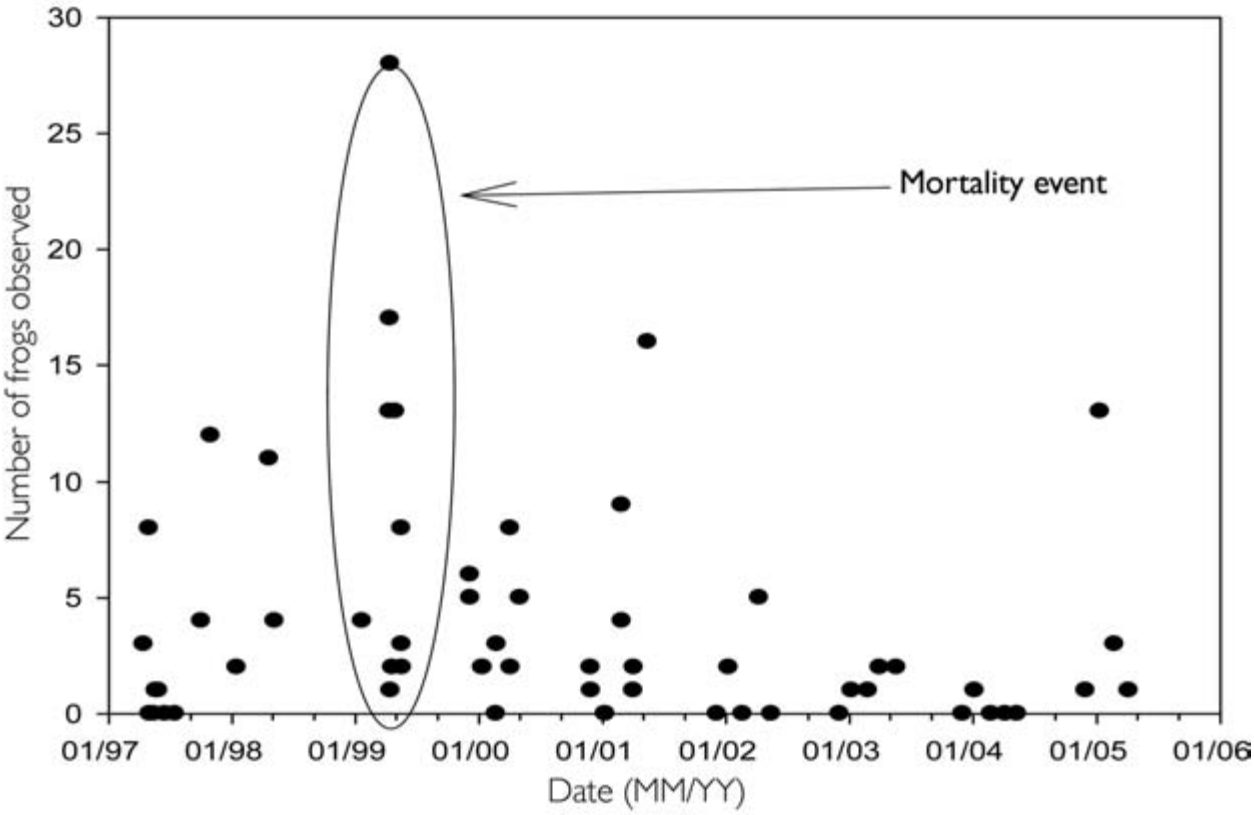

Figure 2. Numbers of live frogs recorded during the Green and Golden Bell Frog monitoring program in the GE complex. Points represent discrete survey nights but appear to overlap where surveys occurred on successive nights.

Discussion

This is the first published observation of a mortality event directly attributed to the chytrid fungus for a wild population of the Green and Golden Bell Frog. Results of ongoing monitoring indicate that if a decline in numbers occurred, the population was able to recover following this mortality event. It is not clear why only a small area of the site was affected by the disease when no other events were recorded in adjacent areas or in subsequent monitoring of the site. Data suggest that frogs readily move between pond complexes at the site (AMBS Consulting 2001). The area affected is similar to others in Sydney Olympic Park in the aquatic and terrestrial habitat characteristics and the physio-chemical properties (pH, dissolved solids, temperatures, turbidity) of the water. Data relating to these conditions are presented in AMBS Consulting (2001). In addition, these ponds were exposed to the same management actions as other ponds that weren't affected (AMBS Consulting 2001).

There is little doubt that chytrid can and does affect individuals of the Green and Golden Bell Frog. While until now there has been no published data on this fact (although see Stockwell *et al.* 2008), several observations have been made of chytrid related mortality. A discussion of these is presented in the draft recovery plan for this species (DEC 2005). However, what is not clear is the extent to which the disease can affect wild populations and whether it has been the cause of extinction in other populations.

In the case of the Sydney Olympic Park, the Green and Golden Bell Frog population has remained at a similar population size following the mortality event (AMBS Consulting 2001). The means by which this has occurred is not known, however, there are several possibilities. One possibility is that the protection of large areas of habitat across the site may have allowed the population to recolonise the GE complex in subsequent seasons. At this site, marked frogs are rarely recaptured in subsequent seasons and therefore it cannot be determined whether or not this occurred. However, marked frogs were recorded moving into the GE complex from other parts of the site in subsequent years of monitoring (AMBS Consulting 2001). Alternatively, the population in the GE complex remained stable despite the mortality event without any immigration from surrounding areas. It is not clear why only one group of ponds was affected and none of the other pond complexes. Survey effort was consistent over the site at the time of the mortality event and it is likely that if other areas were affected at least some dead or dying individuals would have been encountered. During the mortality event, healthy frogs were observed across the site including within the GE complex. Further research is needed to understand the distribution and behaviour of chytrid fungus within wild populations of the Green and Golden Bell Frog.

Most of the remaining Green and Golden Bell Frog populations are under active management in NSW. The mortality event recorded here suggests that chytrid fungus could have a rapid impact on a small isolated population, although the results indicate that the impact may not be as significant in larger populations spread across interconnected habitats. Management of populations should therefore aim to maximise the variety and number of ponds available to the Green and Golden Bell Frog. Similarly, connectivity should be maintained between these ponds to allow recolonisation if a mortality event occurs.

The data presented here indicate a single mortality event within an otherwise apparently healthy population of the Green and Golden Bell Frog. In this case, the Sydney Olympic Park population and the sub-population residing in the GE complex have been able to survive the mortality event. However, chytrid may still pose a significant challenge for managers of populations of this species, in particular small isolated populations. Research should be conducted to determine the extent to which chytrid is present within extant populations of the Green and Golden Bell Frog. In addition, research is needed to determine whether there are means of controlling outbreaks of the disease within populations.

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